NOVEL CYTOTOXIC AND SELECTIVE THIONAPHTHOQUINONE DOWNREGULATES MMP-2 AND MMP-9 EXPRESSION AND INHIBITS MIGRATION OF CERVICAL CANCER CELLS

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Introduction

From 1981 to 2019, 49,5% of the approved anticancer drugs were natural products or structurally related to natural products, demonstrating the importance of identifying new bioactive compounds in nature[1]. Naphthoquinones are a large class of secondary metabolites that are produced by several living organisms[2]. Lawsone, a dye isolated from *Lawsonia inermis*, is a well-known natural occurrence naphthoquinones[3]. In medicinal chemistry, the addition of sulfur heteroatoms to the naphthoquinone nucleus can amplify pharmacological properties such as anticancer activity[4]. The mechanisms by which naphthoquinones exert their biological activities are most often linked to their ability to stimulate the production of reactive oxygen species (ROS), but are not limited to it[5]. Cervical cancer is the 4th more incident and 3rd more lethal type of cancer among women in Brazil[6], which highlights the importance of finding novel effective drugs against this type of tumor. In addition to proliferation, migration and invasion are important hallmarks of cancer progression. In this context, metalloproteinases (MMPs) digest the basement membrane, making invasion and metastasis possible for cancer cells. This study aimed to evaluate the biological activities of novel thionaphthoquinones derived from lawsone against cervical cancer cells and identify their possible molecular mechanisms.

Material and Methods

Lawsone-derived thionaphthoquinones (TNQs) were synthesized via a microwave reaction and isolated via column liquid chromatography. Their structures were elucidated by mass spectrometry, ultraviolet spectroscopy, and nuclear magnetic resonance. The IC_{50} values of the TNQs were determined using an MTT viability assay. The selectivity index was calculated as the ratio between the IC_{50} in non-tumor cells (NIH-3T3) and the IC_{50} in tumor cells (HeLa). An MTT viability assay was also performed with n-acetyl-cysteine pretreatment for 2 h before applying the treatments to investigate the effects of ROS production on cell viability. The antimigratory activity was assessed for 48 h using a wound-healing assay with HeLa cells at sub-lethal concentrations. The expression of matrix metalloproteinases (MMP) 2 and 9 was measured by dye-based RT-qPCR. The molecular mechanisms of the TNQs were investigated using *in silico* target fishing in PharmMapper software and molecular docking using Autodock4 software. Absorption, distribution, metabolism, and excretion (ADME) properties were predicted using SwissADME software. Carboplatin and/or doxorubicin were used as positive controls. IC_{50} values were obtained by non-linear regression in a dose-response curve, and analysis of variance (ANOVA) followed by Dunnett's test was conducted to compare the compounds with an untreated group, using GraphPad Prism 8.

Results and Discussion

Five novel TNQs were synthesized in this study. TNQ10, the most potent, had an IC_{50} value of 25.1 µM, whereas the control carboplatin had an IC_{50} of 61.9 µM. TNQ10 and carboplatin showed selectivity indices in NIH-3T3 cells of 3.49 and 3.72, respectively. These results indicate that TNQ10 is more potent and has selectivity comparable to of that the standard treatment for cervical cancer carboplatin.

TNQ10 inhibited the migration of HeLa cells at 1 µM after 24 h, whereas carboplatin did not inhibit the migration at 5 µM. TNQ10 was capable of inhibiting HeLa migration at a concentration 25-fold lower than the IC_{50}, whereas carboplatin was not capable of doing so even at only a 12-fold lower concentration than its IC_{50}.

TNQ10 downregulated MMP-2 and MMP-9 mRNAs expression by 72% and 55%, respectively, whereas carboplatin upregulated their expression by 38% and 127%, respectively. In addition to migration, another important prognostic marker is cancer invasion. In this context, metalloproteinases are essential enzymes that digest the basal membrane to allow cancer cells to invade adjacent tissues. TNQ10 significantly reduced MMP-2 and MMP-9 expression, whereas carboplatin increased MMP expression. MMP downregulation, in addition to antimigratory activity, suggests that TNQ10 might inhibit the migration and invasion of cervical cancer cells, whereas carboplatin might not have an impact on such activity or even enhance the tumoral ability to do so.

Target fishing indicated that the most likely molecular target of TNQ10 is JNK1. After redocking validation, molecular docking with JNK1 (PDB ID= 4LF7) resulted in binding energy for TNQ10 of -10.61 and -7.39 kcal/mol for the control AX1387, which suggests that TNQ10 could bind in a more efficient way than the crystallographic ligand AX1387. Naphthoquinones can induce cancer cell death by modulating the JNK/p38 pathway; however, this modulation is typically mediated by ROS production[7]. Pretreatment with the antioxidant NAC did not protect the cells from the cytotoxic effects of TNQ10, indicating that its effects might not be related to ROS production.

Finally, ADME studies indicated that TNQ10, although highly lipophilic, passes on Lipinski and Muegge's rules-of-thumb in the drug discovery industry.

Conclusion

In this study, we describe the biological activity of TNQ10, a novel potent and selective cytotoxic thionaphthoquinone that can inhibit HeLa cell migration and downregulate the expression of matrix metalloproteinases, potentially mediated by interaction with JNK1. These findings indicate that TNQ10 is a promising compound for further advancement in the anticancer drug discovery pipeline.

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